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Efficacy of Terramycin® 200 for Fish (Oxytetracycline Dihydrate) for the Skeletal Marking of Rainbow Trout *Oncorhynchus mykiss*

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In the USA, the only chemical compounds currently approved by the U.S. Food and Drug Administration (FDA) for the skeletal (fluorescent) marking of fish that might enter the human food chain are oxytetracycline hydrochloride (OTC-HCL) and oxytetracycline dihydrate (OTC-DH). Oxytetracycline-HCL is approved for the skeletal marking of all finfish fry and fingerlings via static-bath immersion. Oxytetracycline-DH is approved for the skeletal marking of Pacific salmon via oral administration in feed.

Terramycin® 200 for Fish Type A Medicated Article (TM200; Phibro Animal Health, Corp., Ridgefield Park, New Jersey USA) is the only approved OTC-DH product currently sold in the USA for use in finfish. Terramycin® 200 (44.1% active OTC-DH) can be purchased over-the-counter and top-coated onto fish feed, or TM200-treated fish feed can be purchased from a licensed feed mill. The currently approved skeletal marking claim is to administer TM200 in feed to Pacific salmon at 250 mg OTC per kg fish per d for 4 d. Use is restricted to fish less than 30 g, and there is a 7-d posttreatment withdrawal period. The U.S. aquaculture community would like to amend this claim to read as follows:

> *Administer TM200 in feed at 3.75 g OTC per 100 lb fish per d (82.7 mg OTC/kg fish/d) for 10 d for the skeletal marking of all freshwater-reared salmonids less than 55 g (21-d withdrawal period).*

The prospective amended claim would increase the number of salmonid species and maximum size of fish that could be legally marked, while decreasing the amount of OTC administered per treatment event. The amended claim would also increase the withdrawal period; however, such a change in withdrawal time will not adversely affect skeletal marking programs because of the inherent withdrawal period associated with treating small fish. Finally, the treatment regimen administered under the amended marking claim would be the same as the treatment regimen allowed under several currently approved therapeutic claims for TM200.

In this bulletin, we summarize a study conducted to support FDA approval of the proposed amended TM200 skeletal marking claim. In the study, rainbow trout served as a representative freshwater-reared salmonid species. To demonstrate efficacy, the study protocol specified that the mean percentage of rainbow trout with marked vertebrae in TM200-treated tanks had to be equal to or greater than 70%.

Methods

The study was conducted February - April 2009 at the U.S. Fish & Wildlife Service, Bozeman Fish Technology Center, Bozeman, Montana USA. Test fish were healthy rainbow trout fingerlings (initial mean body weight, 37 g), which had been obtained as eyed eggs from Troutlodge, Inc. (Tacoma, Washington USA facility). Terramycin® 200-treated feed (Bio-Trout 3.0 mm; 1% TM200) and control feed (Bio-Trout 3.0 mm) were obtained from Bio-Oregon (Longview, Washington USA). Feed samples were analyzed by Eurofins, Inc. (Portage, Michigan USA) to determine the OTC-DH concentrations in the treated and control feeds.

Completely randomized design procedures were used to transfer 180 fish from a reference population to nine test tanks (six treated and three control; 20 fish per tank) and assign a treatment condition (TM200-treated or nontreated control) to each tank. The in-life phase comprised a 1-d acclimation period, 10-d treatment period, and 22-d posttreatment period. No feed was administered during the acclimation period. During the treatment period, TM200-treated feed was administered to the treated tanks and control feed was administered to the control tanks at 1.875% initial mean body weight per day. Control feed was administered to all tanks during the posttreatment period, and daily feed amounts were adjusted for growth twice during the posttreatment period. Data collected daily included general fish behavior, feeding (appetite) behavior, mortality, water temperature, and dissolved oxygen concentration. Source water hardness, alkalinity, and pH were measured three times. The in-life phase of the study was single-masked such that personnel

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administering feed and collecting data did not know which tanks were being treated.

At the end of the in-life phase, all test fish were removed from test tanks, euthanized in a solution of tricaine methanesulfonate, individually bagged by fish and tank number, and frozen whole. Approximately 1 month later, test fish were removed from the freezer and thawed. Two vertebrae were extracted from each fish, and each vertebra was cleaned to remove all soft tissue. Each vertebra was then examined for a fluorescent OTC-DH mark under an ultraviolet light (~365 nm wavelength) and dissecting scope (8 - 40X magnification). Mark quality was graded 0 (no mark), 1 (faint and incomplete mark circle), 2 (faint and complete mark circle), or 3 (bright and complete mark circle). Fish with vertebral marks graded 0 or 1 were classified as "not marked," whereas fish with vertebral marks graded 2 or 3 were classified as "marked." The study participant who examined vertebrae and graded OTC-DH marks did not know which tanks of fish had been treated.

Results and Discussion

Feed sample analysis revealed that an average dose of 3.33 g OTC-DH per 100 lb fish per d had been administered to treated tanks. This dose was only 89% of the target; however, it was within the acceptable range specified in the study protocol. A small amount of OTC-DH was detected in control feed; consequently, an average dose of 0.01 g OTC-DH per 100 lb fish per d had been administered to control tanks.

Mean daily water temperature was 10.3°C (range, 9.8 - 11.0°C), and mean daily dissolved oxygen concentration was 8.2 mg per L (range, 7.3 - 9.6 mg per L). Mean source water hardness, alkalinity, and pH were 293 mg per L (as $CaCO_3$), 195 mg per L (as $CaCO_3$), and 7.9, respectively. All of these water quality parameters were suitable for rearing healthy rainbow trout.

Throughout the in-life phase of the study, general fish behavior was characterized as normal, feeding behavior was characterized as aggressive, and there was no mortality. Although the average OTC-DH dose administered to the treated tanks was less than the target dose, all of the fish in the treated tanks were classified as "marked," and all of the vertebrae examined from these fish were determined to have a grade 3 mark (Figure 1). Although a small amount of OTC-DH was detected in the control feed, all of the fish in the control tanks were classified as "not marked," and all of the vertebrae examined from these fish were found to have a grade 0 mark (Figure 2). Therefore, in this study, we concluded that TM200 administered in feed at a target of 3.75 g OTC-DH per 100 lb fish per d for 10 d was effective for the skeletal marking of fingerling rainbow trout.

Acknowledgments

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Figure 1. Fluorescent mark on vertebra of a rainbow trout administered Terramycin® 200 for Fish-treated feed at 3.75 g OTC-DH per 100 lb fish per d for 10 d .

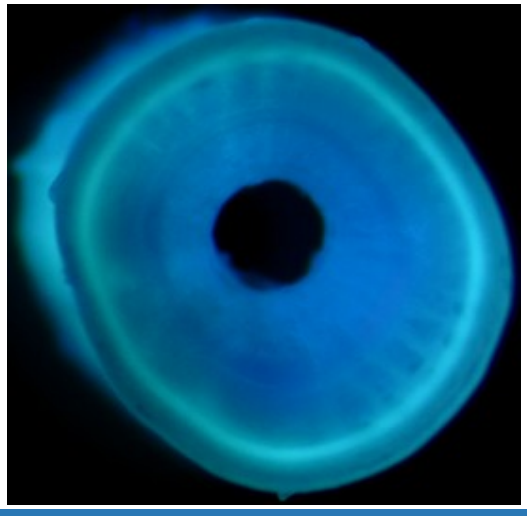

Figure 2. Vertebra of a rainbow trout administered nontreated control feed. Note the lack of a fluorescent mark.